Confirmation of the southern-most population of *Juniperus seravschanica* in Oman by DNA sequencing of nrDNA and four cpDNA regions.

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ABSTRACT

DNA sequencing of nrDNA, plus four cp DNA regions: petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF of putative *J. excelsa* subsp. *polycarpos* from Oman, identified the taxon as *J. seravschanica* which is closely allied with northern populations of *J. seravschanica* in Iran, Pakistan and Kazakhstan. The Oman population, the southern-most population of *J. seravschanica*, may be a Pleistocene relict or the result of more recent long-distance dispersal. Additional populational study will be needed to distinguish between these scenarios. Published on-line **www.phytologia.org** *Phytologia 96(2): 218-224 (July 1, 2014).* ISSN 030319430

KEY WORDS: *Juniperus seravschanica, J. excelsa, J. polycarpos* var. *polycarpos, J. polycarpos* var. *turcomanica,* Oman, DNA sequencing, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF.

Juniperus excelsa M.-Bieb. grows from Greece to Turkey and perhaps as far east as Azerbaijan (Fig. 1). Farjon (2005, 2010) treated *J. polycarpos, J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* as *J. excelsa* subsp. *polycarpos*. However, Adams and Schwarzbach (2012) and Adams (2013), utilizing DNA sequence data, recognized *J. excelsa* in addition to *J. polycarpos* var. *polycarpos*, *J. p.* var. *turcomanica* and *J. seravschanica.* Adams and Hojjati (2012) and Adams, Hojjati and Schwarzbach (2014), using sequences from 4 gene regions, failed to verify the occurrence of *J. excelsa* in Iran, but did find *J. polycarpos, J. p.* var. *turcomanica* and *J. seravschanica* in Iran (Fig. 1). Putative *J. excelsa* from Qushchi, in extreme northwest Iran, had none or only one SNP difference compared with *J. polycarpos* var. *polycarpos* from Armenia and was concluded to be *J. polycarpos* (Adams and Hojjati, 2012).

It is difficult to distinguish *J. excelsa*, *J. polycarpos* and *J. seravschanica*. The distribution of *J. excelsa* into Armenia, Azerbaijan and Iran has proved difficult to determine by modern methods of DNA sequencing, due to the lack of access to these regions. Recently, materials were obtained of *J. excelsa/ J. polycarpos* from Lebanon and *J. excelsa/ J. polycarpos* from Azerbaijan. Adams et al. (2014) found that putative *J. excelsa* from Azerbaijan was *J. polycarpos,* and unusual trees of putative *J. excelsa* at El Njass and Aarsal, Lebanon (Douaihy et al, 2011; 2013), was identified as *J. polycarpos* using DNA sequence data (Fig. 1).

In the Flora of the Arabian Peninsula and Socotra (Miller and Cope, 1996), *Juniperus excelsa* M.-Bieb. subsp. *polycarpos* (K. Koch) Takht. (treated as *J. polycarpos* K. Koch in Adams, 2014) is listed as growing in the western Al Hajar Mountains, southwest of Muscat at 1450-3000 m. Due to the proximity of the Hajar mountains to *J. seravschanica* in the Khabr mountains in Iran (Fig. 1), it seemed the juniper from Oman might be *J. seravschanica*. The purpose of the paper is to examine nrDNA, and 4 cp DNA

regions: petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF to establish the identity of the Al Hajar juniper of Oman.

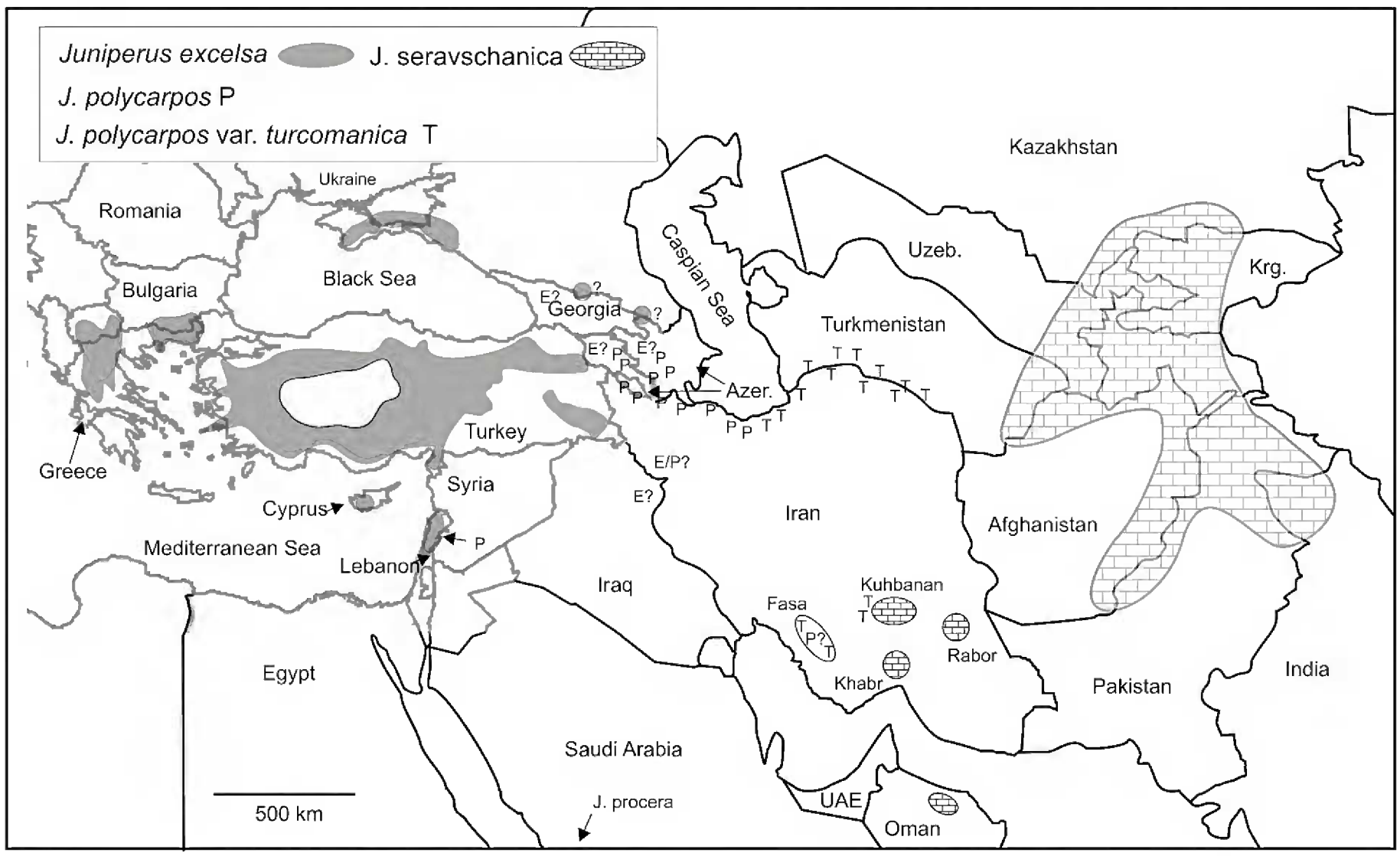

Figure 1. Distribution of *J. excelsa*, *J. polycarpos* var. *polycarpos* (P) and *J. p.* var. *turcomanica* (T) . Questionable locations of *J. excelsa* and *J. polycarpos* are indicated by E? and P? (modified from Adams, 2014).

MATERIALS AND METHODS

J. excelsa: Eskisehir, Turkey, 820m, *Adams 9433, 9435,* Lemos, Greece, 1100m, *Adams 8785, 8786*, Afqa, Lebanon, 1306 m, *Adams 14155-14157*, (=*Bouchra Douaihy 1-3*), 34° 04' 58.12"N, 35° 53' 08.52"E, 4 Nov 2013,

J. polycarpos: Armenia, Lake Sevan, 1900m, *Adams 8761-8763*; Azerbaijan, 177-231m, *Adams 14161-14162* (=*Vahid Farzaliyev 1-10*), 40° 44' 41.05" N; 47° 35' 19.14" E, Dec 2013; Lebanon, Wadi El Njass, 2287m, *Adams 14158-14160*, (=*Bouchra Douaihy 4-7)*, 34° 20' 47.79"N, 36° 05' 45.54"E, 14 Nov 2013; Fasa, Iran, *Adams 13756*, *Hojjati*,

J. polycarpos var. *turcomanica*: Kopet Mtns., Turkmenistan, *Adams 8757, 8758*; Fasa, Iran, *Adams 13757, 13758* (=*Hojjati ns*), Oct. 2012, 29° 09' 57.8" N, 53° 40' 7.8" E,

J. procera: Guder, Ethiopia, *Adams 6184-6188*,

J. seravschanica: Oman: *Amina Al-Farsi, 562-564*, *RPA* lab acc. *14203-14205*, 23° 07' 41" N, 57° 36' 9.3" E, 2340 m, northern Hajar Mtns., Jebel Al Akhdar, Da'an Al Pesaiteen; Dzhabagly, Kazakhstan, *Adams 8224-8226*; Pakistan, *Adams 8483-8485*; Kuhbanan, Iran *Adams 13760, 13761,* (=*Hojjati ns*), Oct. 2012, 31° 28' 21.5" N, 55° 52' 58.9" E; Rabor, Iran, *Adams 13775, 13777*, (=*Hojjati ns*), Oct. 2012, 28° 49' 06.7" N; 56° 21' 21.7" W. elev. 2086 m; Khabr, Iran, *Adams 13771,13772* (=*Hojjati ns*), Oct. 2012, 28° 51' 8.4" N, 56° 22' 51.7" E, possible hybrid (Adams, Hojjati and Schwarzbach (2014),

J. virginiana (out-group): Knoxville, TN, USA, *Adams 10231, 10232.*

Voucher specimens deposited in the Herbarium, Baylor University (BAYLU) and Sultan Qaboos University (SQU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from http://www.geneious.com/), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion.

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS) and four cp regions (petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF) yielded 4430 bp of data. The Bayesian consensus tree (Fig. 2) shows *J. seravschanica, J. polycarpos, J. p.* var. *turcomanica, J. procera* and *J. excelsa* in well-supported clades. The Oman junipers are in a well-supported clade with *J. seravschanica*, nested close to Khabr and Rabor, Iran (Fig. 2). Khabr is the nearest population of *J. seravschanica* to Oman, and Rabor is the next nearest population (Fig. 1). It seems likely that during the last Pleistocene ice advance (ending ~ 15k ybp), populations of *J. seravschanica* in this region expanded their ranges into lower, cooler areas. If so, the range of *J. seravschanica* may have been nearly continuous from Pakistan to Rabor, to Khabr. Thence by long-distance dispersal (common for the establishment of *Juniperus* on islands, Adams, 2014) it became established in the Al Hajar Mtns. of Oman. Kurschner (1998) listed the Omani *Juniperus* among some other taxa of Irano-Turanian origin and described them as invaders in Arabia. Of course, the population in the Al Hajar Mtns. may be a relict from an ancient distribution. Additional studies comparing the genetic structure of *J. seravschanica* populations from Pakistan, southern Iran and Oman will be needed to determine if the Oman population is relictual or a more recent population, established by long distance dispersal.

Juniper trees in this region have the typical round to pyramidal shaped crowns (Fig. 3) of *J. seravschanica*. Even at high elevations (e.g., 2340 m at collection site), the area is semi-desert (Fig. 3) with very low precipitation. Pollen appears to be shed in Dec - Feb, mainly because one can see very small (either recently or not yet pollinated) seed cones (insert, Fig. 4). Adams (2014) notes that pollen is shed in *J. seravschanica* in fall-winter. However, those observations were based on field notes from his Kazakhstan collections. It is likely that Oman junipers shed pollen somewhat later than found in the much colder region of Kazakhstan.

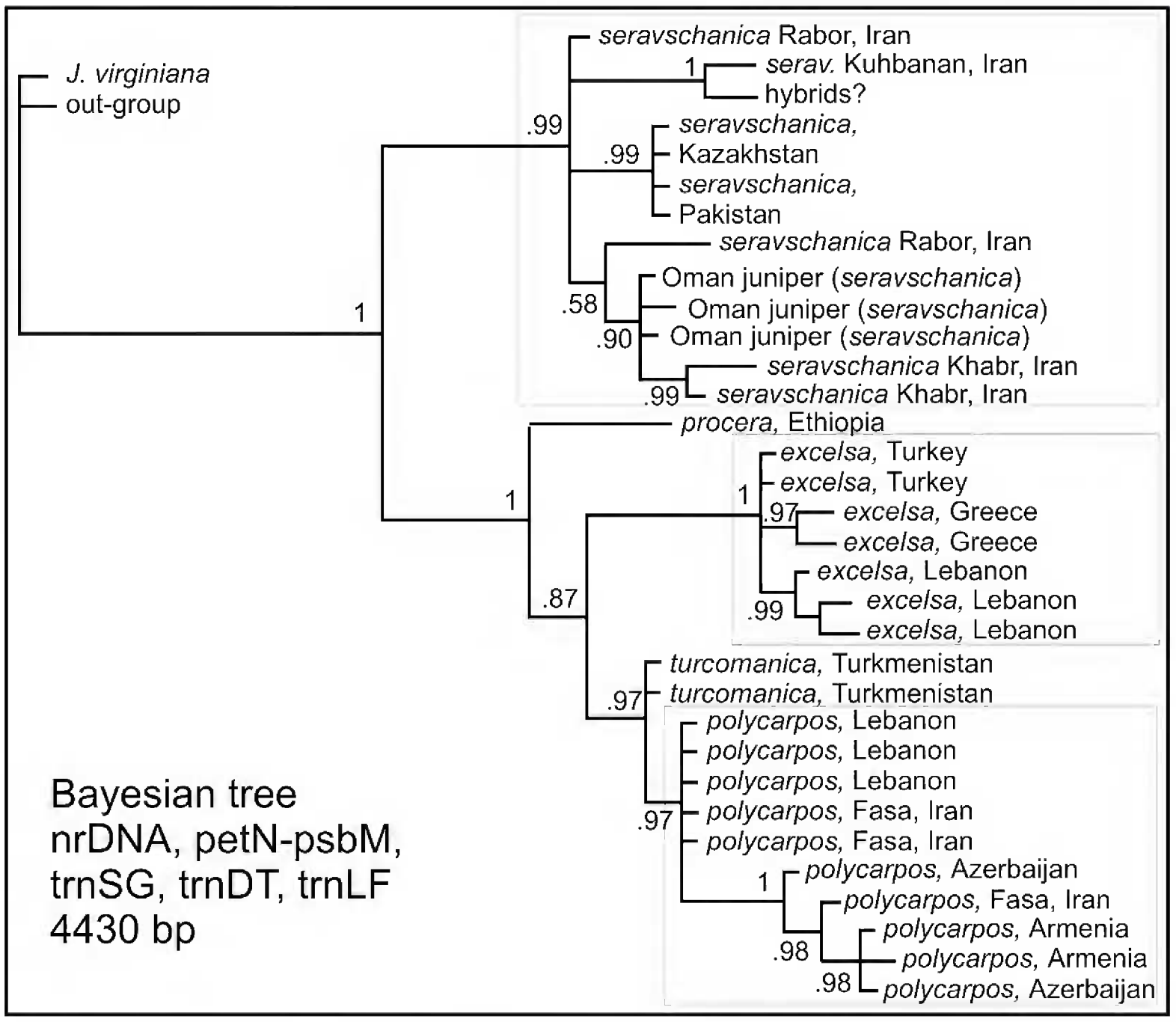

Figure 2. Bayesian tree based on nrDNA (ITS) and four cp regions: petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF (4430 bp). Numbers at the branch points are posterior probabilities. Boxes enclose *J. seravschanica*, *J. excelsa* and *J. polycarpos*. The Oman junipers are grouped with *J. seravschanica*.

Figure 3. *Juniperus seravschanica*, Al Hajar Mtns., Oman. The species is locally common (see Fig. 5).

Figure 4. Leaves and seed cones on *J. seravschanica*. Note the small, new seed cones (inset). Photo taken on 19 Feb. 2014. Pollination appears to have occurred in the winter (Dec. - Feb.).

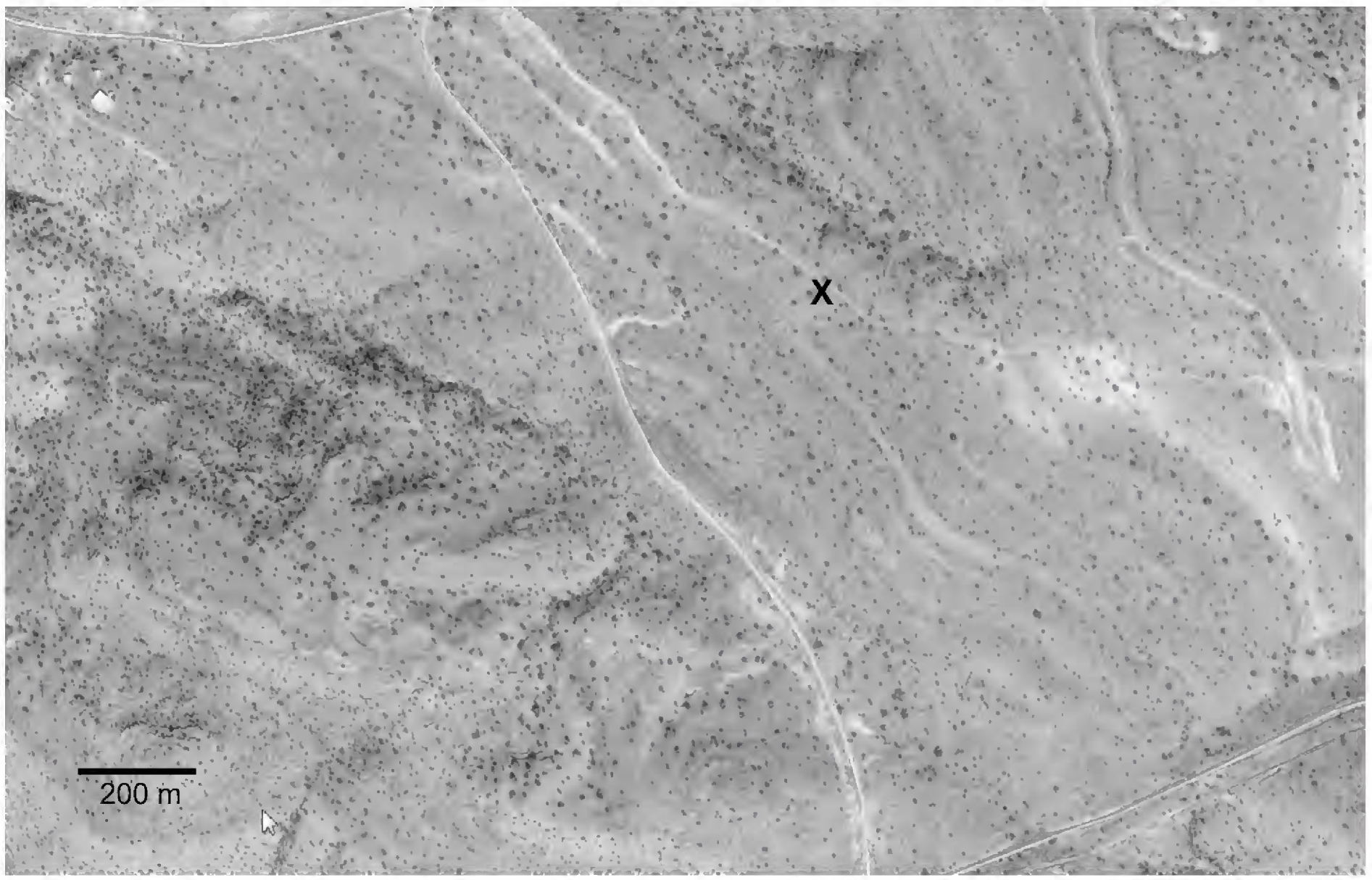

Figure 5. Satellite photo of the population of *J. seravschanica* in the Al Hajar Mtns., sampled for this study. X marks the area where trees were sampled. Nearly all the trees in the photo are *J. seravschanica* (photo courtesy of Google Earth).

The exact distribution of *J. seravschanica* is not known. Plotting specimens from Kew and ON shows (Fig. 6) that all these collections were from an area only about 100 km long. However, as seen in the satellite photo (Fig. 5), the taxon can be rather common in this region. Additional field explorations will be needed to fully map the range of *J. seravschanica* in the Al Hajar Mtns. of Oman.

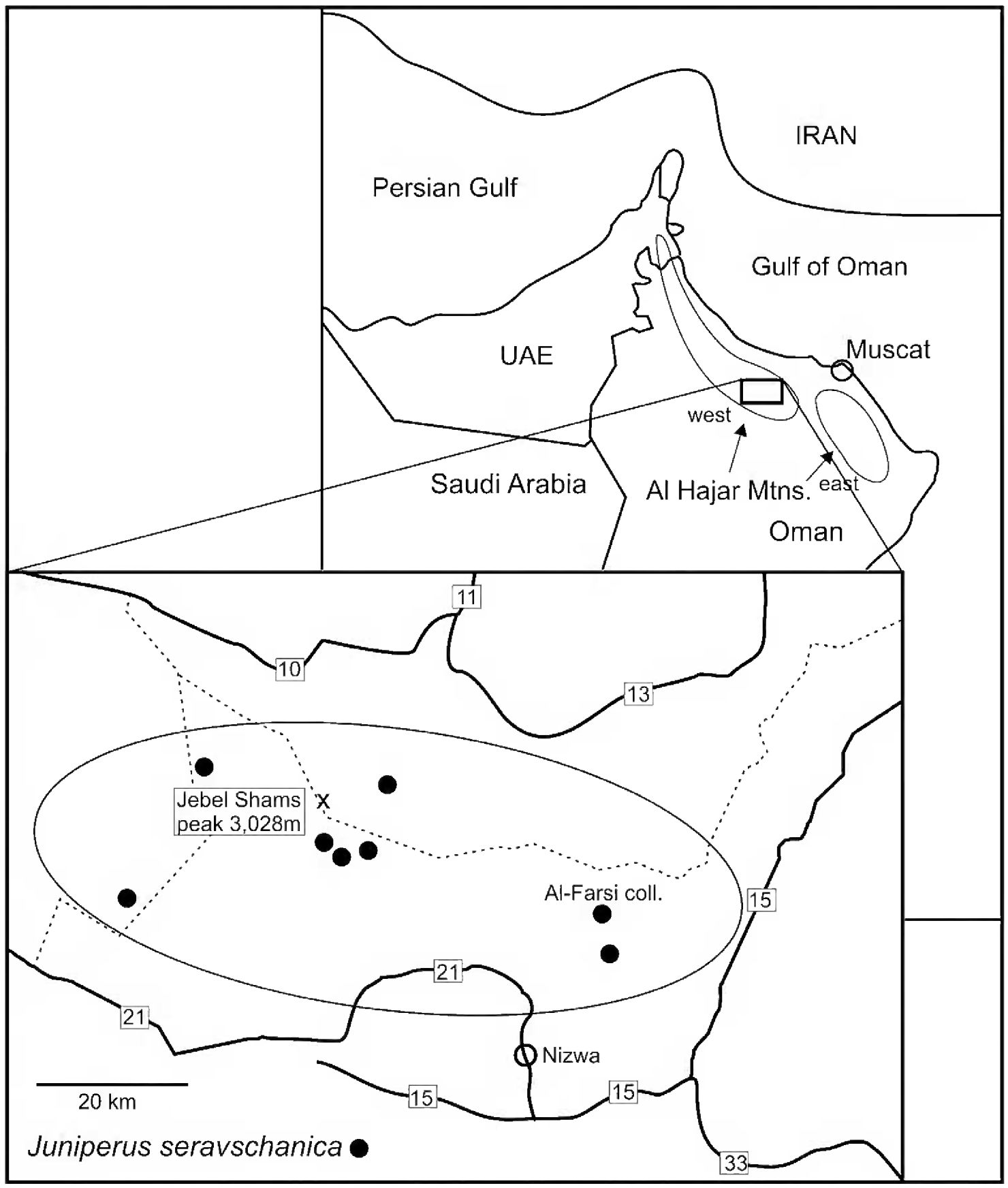

Figure 6. Distribution of *J. seravschanica* mapped from specimens at Kew and ON. The location of the collection (by A. Al-Farsi, 2/2014) for this study is noted.

ACKNOWLEDGEMENTS

Thanks to Tonya Yanke for lab assistance. This research was supported in part with funds from Baylor University.

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